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YELLOW FEVER INSTITUTE, BULLETIN No. 15.
*Treasury Department, U. S. Public Health and Marine-Hospital Service.*
WALTER WYMAN, *Surgeon-General.*

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# REPORT

OF

# WORKING PARTY NO. 3,

# YELLOW FEVER INSTITUTE.

## ATTEMPTS TO GROW THE YELLOW FEVER PARASITE.

## THE HEREDITARY TRANSMISSION OF THE YELLOW FEVER PARASITE IN THE MOSQUITO.

BY

M. J. ROSENAU

AND

JOSEPH GOLDBERGER.

JANUARY, 1906.

WASHINGTON:
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Section B.—ETIOLOGY. P. A. Surg. M. J. ROSENAU, Chairman of Section.

# ATTEMPTS TO GROW THE YELLOW FEVER PARASITE.

# THE HEREDITARY TRANSMISSION OF THE YELLOW FEVER PARASITE IN THE MOSQUITO.

By M. J. Rosenau, *Passed Assistant Surgeon,*
and
Joseph Goldberger, *Passed Assistant Surgeon,*
*U. S. Public Health and Marine-Hospital Service.*

## ATTEMPTS TO GROW THE YELLOW FEVER PARASITE.

In view of the fact that the infective principle causing yellow fever may pass through the close-grained pores of a Pasteur-Chamberland B filter, it seemed to us hopeless, with the limitations of the present microscope, to expect to see the causative agent of this disease by direct examination of the blood.

Novy's work with trypanosomes, both his success in their artificial cultivation and his filtration experiments indicating the possibility of an "ultramicroscopic" phase in their developmental cycle, suggested to us the possibility of cultivating by similar methods the yellow fever parasite and thus perhaps developing a stage in its life cycle which might readily be visible. We attempted, therefore, to grow the parasite of yellow fever in the "water of condensation" of blood-agar tubes.

The culture tubes were prepared in several different ways; both human blood (nonimmune) and rabbit's blood were used. Some tubes were prepared with the whole, and some with the defibrinated blood. When the whole blood was used it was quickly added while fresh to melted agar, at 42° C., in the proportion of about 2 parts of blood to 1 of agar. After mixing, the tubes were slanted and allowed to set. The defibrinated blood was prepared by whipping in the usual way, and then it was added to the melted agar in about the same proportions as above stated. After standing a short while the blood-agar slants, thus prepared, developed from several drops to about 1 cc. of "water of condensation."

A drop or two of blood from typical cases of yellow fever taken from the arm vein during the early stages of the disease was planted into this "water of condensation."

Other cultures were prepared with much larger quantities of the yellow fever blood, using the yellow fever blood itself as a part of the culture medium. To tubes containing about 2 cc. of melted agar, about 4 cc. of fresh yellow fever blood was added as quickly as possible after its withdrawal from the vein. Other tubes were similarly prepared with the defibrinated yellow fever blood.

Some of the cultures were kept in the incubator at 37° C., and others at room temperature. Of the latter, some were kept in the dark and others exposed to the light in order to simulate the conditions as they appear to occur in the mosquito.

The water of condensation was examined from time to time both in hanging drop and in stained smears. It was found to contain large numbers of bodies of various sizes and shapes, some of them exhibiting curious and exaggerated Brownian motion. All of the particles were interpreted as degeneration products, mostly from the cellular elements of the blood.

# HEREDITARY TRANSMISSION OF THE YELLOW FEVER PARASITE IN THE MOSQUITO.

The question of the herditary transmission of the yellow fever parasite from the *Stegomyia fasciata* to its progeny is of interest both biologically and practically.

Reasoning by analogy such transmission can not be regarded as impossible, as it is known to occur in some, probably closely allied diseases, namely through the tick in Texas fever[a] and canine piroplasmosis[b]. Schaudinn[c] has satisfied himself that hereditary transmission of the tertian malarial parasite (*Plasmodium vivax*) occurs in *Anopheles*, and recently Dutton and Todd[d] have shown that the spirochaete of the tick fever of the Congo is passed from tick to tick through the egg.

To the sanitarian the question is of interest in its bearing on the problem of the recrudescence of yellow fever.

The recrudescence of epidemics of yellow fever has heretofore been explained in one of two ways: (1) Either a *Stegomyia fasciata* that had become directly infected by feeding on a case of the disease had survived (as they are experimentally known to be able to do), or (2) the infection had in the interval between the epidemics been continued by unrecognized cases and on the recurrence of favorable conditions the disease would reassume epidemic proportions.

From time to time a third explanation has been advanced, namely, the transmission of the infection from the mother mosquito through the eggs to its progeny[e] which, under favorable circumstances, were capable without themselves directly having had access to a case of yellow fever, of giving the disease to a susceptible individual. This explanation has now received the support of an experimental case of yellow fever induced by the sting of a *Stegomyia fasciata* apparently hereditarily infected. This case is reported by Marchoux and Simond in a paper on "The hereditary transmission of the yellow fever virus in the *Stegomyia fasciata*," a translation of which we give in appendix, p. 114.

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[a] Smith and Kilborne, 1893, U. S. Department of Agriculture, Bureau of Animal Industry, Bulletin No. 1.

[b] Lounsbury, C. P., Transmission of Malignant Jaundice of the Dog by a Species of Tick. Reprint Cape of Good Hope Agricultural Journal, November 21, 1901.

[c] Schaudinn, Fritz, Generations und Wirtswechsel bei Trypanosoma und Spirochaete. Arbeiten aus dem Kaiserlichen Gesundheitsamte, Berlin, Band XX, Heft 3, 1904, p. 428.

[d] Dutton and Todd, Liverpool School of Tropical Medicine, Memoir xvii, 1905.

[e] Finlay (Medical Record, N. Y., May 27, 1899, p. 738), inspired by the discovery of Smith and Kilborne of the hereditary transmission of the parasite of Texas fever in the tick, was the first to suggest the hereditary transmission of the yellow fever parasite in the mosquito. In 1901 Reed, Carroll, and Agramonte (American Medicine, Phila., July 6, 1901, p. 19) reported the negative results of an inoculation experiment with 14 mosquitoes hatched from the ova of a *Stegomyia fasciata* that had already shown itself capable of conveying the disease, and Guiteras (American Medicine, Phila., Nov. 23, 1901, p. 810) reports two experiments. In both the mother

Against this one positive case we submit the negative results of inoculations attempted in 13 nonimmunes.

In an endeavor to account to ourselves for the divergence of our results from that of the French workers we have considered several factors in the problem, failure to comply with any of which might readily be productive of negative results. We believe, however, that our work, so far as we can judge from the details in their paper, closely parallels the work of Marchoux and Simond. Naturally the first factor which arises for consideration is whether the mothers of the *Stegomyia fasciata* used by us for the inoculation of our nonimmunes were infected. Marchoux and Simond state that the mother of the insect whose sting produced the positive case of fever had been made to feed on *several* (number not stated)[a] of their

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mosquitoes were experimentally proved to be infected. In the first the progeny of one mother were tried without results upon three nonimmunes; in the other a large brood from several mosquitoes was kept in a breeding jar and was fed upon nonimmunes with impunity.

[a] During the progress of this bulletin through the press the second report of the French Commission to Rio de Janerio (Marchoux and Simond, Annales de l'Institut Pasteur, Paris, XX, 2, Jan. 25, 1906, p. 16) came to our hands, giving full details of the positive experimental case of yellow fever produced by the sting of a *Stegomyia fasciata* apparently hereditarily infected, as follows:

"Experiment.—A female *Stegomyia fasciata* born in the laboratory and arriving at the adult stage January 19 (*probably means January 9*), 1905, was paired from the 9th to the 11th. It stung a yellow-fever patient presenting a severe attack in the second day of the disease, on the 11th of January, and laid the first batch of eggs January 17. On the 25th of January it stung another patient in the second day of the disease, who presented an attack of moderate severity, and laid the second batch of eggs January 28. This latter batch hatched out from the 3rd to the 4th of February, and the larvæ cared for in the laboratory produced perfect insects February 16.

"Two females from this batch were isolated in tubes and fed with sirup up to March 2. At this date—that is to say, 14 days after completing their metamorphosis, these mosquitoes were made to sting subject A. This subject, a Portuguese, had arrived in Brazil only a few days before and had never had yellow fever. He showed no reaction to this inoculation. After an interval of eight days, March 10, the same subject was stung a second time by one of the two mosquitoes, the other having accidentally died. Four days later, March 14, he presented symptoms of yellow fever."

It will be noted, therefore, that the eggs giving rise to the progeny that apparently caused an attack of yellow fever were laid by the mother mosquito 17 days after the first and 3 days after the second feed of yellow-fever blood. (Compare our mosquitoes Nos. 3 and 4, Group II, of Set 1, p. 107.)

cases of yellow fever in order to determine a heavy infection. In our work mother mosquito of Set 1 had three feedings of yellow fever blood from two severe cases.

Mother mosquito of Set 2 and that of Set 3 each had one feed of yellow fever blood early in the disease.

That the interval between the infecting feed and oviposition must enter as an important element in the transmission of the infection through the eggs to the progeny—if such transmission ever takes place in yellow fever—must be evident. Marchoux and Simond do not report clearly on this point;[a] they state simply that their mosquito was 20 days old at the time of oviposition and that some time prior to the laying it had been made to sting several cases of yellow fever.

In our experiments the intervals were as follows:

### SET 1.

*Group I, mosquitoes Nos. 1 and 2.*—The eggs producing these mosquitoes were laid 13½ days from the first, 11½ days from the second, and about 2½ days after the third feed of yellow fever blood.

*Group II, mosquitoes Nos. 3 and 4.*—The eggs giving rise to this group were laid 16 days from the first, 14 days from the second, and about 5 days from the third feed of yellow fever blood. (Compare footnote ([a]), page 106.)

### SET 2.

*Group I, mosquito No. 5.*—The eggs giving rise to this group were laid 15 days after one feed of yellow fever blood.

### SET 3.

*Group I, mosquito No. 6.*—The eggs giving rise to this group were laid 14 days after one feed of yellow fever blood.

*Groups II, III, and IV, mosquitoes Nos. 7, 8, 9, 10, 11, 12, 13, and 14.*—The eggs giving rise to these groups were laid 15 days after one feed of yellow fever blood.

Another important factor in the problem, and one to which Marchoux and Simond call attention, is the time needed by the hereditarily infected mosquito to become infective. In their case this was twenty-two days. In our work the inoculations were carried up to and including at least the forty-ninth day, as shown in Table 1.

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[a] See note [a] on page 106.

TABLE 1.—*Summary of inoculations of nonimmunes by hereditarily infected (?) mosquitoes—subjects kept under observation at least seven days after each inoculation.*

[The mosquitoes are designated by numbers from 1 to 14; nonimmunes by capital letters.]

| Mosquito. | Stung nonimmune— | Age of mosquito at time of stinging. | Remarks. | Mosquito. | Stung nonimmune— | Age of mosquito at time of stinging. | Remarks. |
|---|---|---|---|---|---|---|---|
| | | *Days.* | | | | *Days.* | |
| No. 10 | B | 2 | No reaction. | Nos. 6 and 10 | G | 26 | No reaction. |
| Nos. 7, 8, and 9 | B | 3 | Do. | No. 14 | [a] L | 26 | Do. |
| No. 2 | A | 6 | Do. | Nos. 5 and 9 | F | 27 | Do. |
| No. 1 | B | 9 | Do. | No. 6 | H | 28 | Do. |
| No. 2 | C | 10 | Do. | Nos. 3 and 4 | G | 29 | Do. |
| No. 2 | A | 12 | Do. | Nos. 12, 13, and 14 | [b] M | 30 | Do. |
| Nos. 1 and 2 | C | 13 | Do. | No. 6 | J | 31 | Do. |
| Nos. 3 and 4 | B | 13 | Do. | Nos. 1, 11, and 12 | B | 32 | Do. |
| Nos. 1 and 2 | B | 20 | Do. | No. 3 | J | 33 | Do. |
| No. 3 | E | 21 | Do. | No. 6 | M | 34 | Do. |
| Nos. 11 and 12 | H | 22 | Do. | Nos. 12, 13, and 14 | G | 34 | Do. |
| No. 4 | E | 23 | Do. | No. 1 | H | 35 | Do. |
| Nos. 13 and 14 | J | 23 | Do. | Nos. 12 and 13 | J | 36 | Do. |
| Nos. 1 and 2 | D | 24 | Do. | No. 3 | [b] L | 37 | Do. |
| No. 6 | F | 24 | Do. | No. 1 | K | 39 | Do. |
| Nos. 5 and 9 | F | 25 | Do. | Nos. 13 and 14 | N | 39 | Do. |
| Nos. 11 and 12 | J | 25 | Do. | No. 12 | N | 40 | Do. |
| No. 13 | G | 25 | Do. | No. 3 | G | 45 | Do. |
| No. 3 | F | 26 | Do. | No. 3 | N | 49 | Do. |

[a] Subject passed from observation after 6 days.
[b] Subject passed from observation after 5 days.

A factor in the problem which is of prime importance and which must always be reckoned with in estimating the value of negative results is the susceptibility to the disease of the subject used for the inoculations. We were careful to select only those who we were satisfied never had had the disease.

Another factor which must be considered is temperature. No mention is made in the paper of Marchoux and Simond at what temperature their mosquitoes were kept. Our mosquitoes were kept under artificial conditions at a temperature of between 80° and 90° F. (See p. 109.)

Many other factors which we shall not discuss undoubtedly enter into and affect this problem.

## DETAILS OF EXPERIMENTS.

In our study we used three sets of *Stegomyia fasciata*, comprising fourteen insects, the progeny of three mothers that had fed on yellow fever. The history of these mosquitoes is as follows:

### *Set 1, Groups I and II, mosquitoes Nos. 1, 2, 3, and 4.*

*Mother mosquito.*—The mother mosquito of this set was one of a number raised from the larval stage in our laboratory, and, without any prior feeding, was made to sting, on October 3, at 5.20 p. m., a patient (Melancon) with a severe case of yellow fever, 14½ hours after the initial chill; about 42 hours later this mosquito was again made to sting the same patient, now about 56 hours after the onset. On October 14, at 3.55 p. m., this insect was made to sting a second yellow fever patient (Kippers), suffering from a severe attack of the disease, about 48 hours after the onset of the first symptoms.[a] In all, then, this mosquito had three feedings of yellow fever blood from two severe cases in the early stages of the disease. This, as well as all our mosquitoes, was kept in a room in which the temperature was artificially kept between 80° F. and 90° F. Four or five times throughout the course of these experiments the temperature in this room fell to 70° for six or eight hours at a time.

*Group I.*—On October 17, at 8.30 a. m. (13½ days from the first, 11½ days from the second, and about 2½ days from the third feed of yellow fever blood), this mosquito deposited eggs from which there were hatched between November 4 (4 p. m.) and November 5 (8.30 a. m.) two[b] adult female *Stegomyia fasciata*, which comprise our Group I of Set 1, and were numbered 1 and 2.

*Group II.*—On October 19, at 4.30 p. m. (16 days from the first, 14 days from the second, and about 5 days from the third feed of yellow fever blood), this same mother insect laid a second batch of eggs from which were hatched between November 7 (8 a. m.) and November 8 (8.30 a. m.) two adult female *Stegomyia fasciata*, which comprise our Group II of Set 1, and were given the numbers 3 and 4.

### *Set 2, Group I, mosquito No. 5.*

*Mother mosquito.*—The mother mosquito of this set was one of a number raised from larvæ and for some time fed on immune blood.

On October 4, at 9 a. m., this insect was made to sting a severe case (Melancon) of yellow fever 30 hours after the initial chill.[c]

*Group I.*—October 19, at 4.30 p. m. (15 days after the feed of yellow fever blood), this insect laid a batch of eggs from which there was hatched between November 7 (8.30 a. m.) and November 9 (8.30 a. m.) one adult *Stegomyia fasciata*, which was given the number 5 and was the only one comprised in this set.

### *Set 3, Groups I, II, III, and IV, mosquitoes Nos. 7, 8, 9, 10, 11, 12, 13, and 14.*

*Mother mosquito.*—The mother mosquito of this set was one of a number of *Stegomyia fasciata* raised from larvæ in our laboratory and, without any prior feeding, was made to sting on October 4, at 9 a. m., a severe case of yellow fever (Melancon) 30 hours after the initial chill.[a]

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[a] On Oct. 8, 10, and 12, it was permitted to fill itself by stinging an immune. After Oct. 16, fed with sirup.

[b] We mention only the mosquitoes that survived and were used for inoculation; others, including males, are ignored in this report.

[c] On Oct. 6, 8, 10, 12, and 15, fed by stinging an immune. After Oct. 16, fed with sirup.

*Group I.*—October 18, at 4.20 p. m. (14 days after the feed of yellow fever blood), this insect deposited some eggs, from which there was hatched November 12, at 9 a. m., an adult female *Stegomyia fasciata*, which formed Group I of this set and was given the number 6.

*Group II.*—October 19, at 4.30 p. m. (15 days after the feed of yellow fever blood), this mother mosquito laid another batch of eggs, from which there were hatched between November 7 (8.30 a. m.) and November 9 (8.30 a. m.) three female *Stegomyia fasciata*, numbered 7, 8, and 9, which form Group II of this set.

*Group III.*—November 12, at 9 a. m., there was hatched an additional adult female *Stegomyia fasciata*, which was numbered 10 and forms Group III of this set.

*Group IV.*—Between November 13 (8.30 a. m.) and November 18 (8.30 a. m.) there were hatched four additional females, numbered 11, 12, 13, and 14, which comprise Group IV of this set.

At stated intervals these fourteen *Stegomyia fasciata*, the progeny of mothers (Sets 1, 2, and 3) that had fed on cases of yellow fever as above indicated, were made to sting nonimmune subjects, and during the intervals fed upon sirup.

Mosquitoes Nos. 1 and 2 (Set 1, Group I) were applied to and stung seven nonimmunes (A, B, C, D, G, H, K) at various invtervals between the sixth and thirty-ninth day after completing their metamorphoses, as shown in Table 2.

TABLE 2.—*Inoculation with mosquitoes Nos. 1 and 2.*

[Nonimmunes are designated by capital letters.]

| Mosquito. | Stung nonimmune— | Age of mosquito at time of stinging. | Mosquito. | Stung nonimmune— | Age of mosquito at time of stinging. |
|---|---|---|---|---|---|
| | | *Days.* | | | *Days.* |
| No. 2 | A | 6–7 | Nos. 1 and 2 | B | 20–21 |
| No. 1 | B | 9–10 | Nos. 1 and 2 | D | 24–25 |
| No. 2 | C | 10–11 | No. 1 | G | 32–33 |
| No. 2 | A | 12–13 | No. 1 | H | 35–36 |
| Nos. 1 and 2 | C | 13–14 | No. 1 | K | 39–40 |

Of these inoculations, four were made after the twenty-second day (the mosquito incubation period in the successful case reported by Marchoux and Simond), namely, at age periods of 24, 32, 35, and 39 days.

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[a] On Oct. 6, 8, 10, 12, and 14, fed by stinging immune. After Oct. 15, fed with sirup.

Mosquitoes Nos. 3 and 4 (Set 1, Group II) were made to sting eight nonimmune subjects (B, E, F, G, J, L, M, and N) ten times at intervals between the thirteenth and forty-ninth day after emergence as adult insects, as shown in Table 3.

TABLE 3.—*Inoculation with mosquitoes Nos. 3 and 4.*

[Nonimmunes are designated by capital letters.]

| Mosquito. | Stung nonimmune— | Age of mosquito at time of stinging. | Mosquito. | Stung nonimmune— | Age of mosquito at time of stinging. |
|---|---|---|---|---|---|
| | | *Days.* | | | *Days.* |
| Nos. 3 and 4 | B | 13–14 | No. 3 | J | 33–34 |
| No. 3 | E | 21–22 | No. 3 | [a] L | 37–38 |
| No. 4 | E | 23–24 | No. 3 | M | 42–43 |
| No. 3 | F | 26–27 | No. 3 | G | 45–46 |
| Nos. 3 and 4 | G | 29–30 | No. 3 | N | 49–50 |

[a] Subject passed from observation at the end of 5 days.

Mosquito No. 5 (Set 2) was made to sting subject F on two separate days; the first time 25 and the second 27 days after reaching the adult stage, 3 and 5 days, respectively, later than in the positive case reported by the French workers as shown in Table 4.

TABLE 4.—*Inoculations with mosquito No. 5.*

[Nonimmune is designated by a capital letter.]

| Mosquito. | Stung nonimmune— | Age of mosquito at time of stinging. | Mosquito. | Stung nonimmune— | Age of mosquito at time of stinging. |
|---|---|---|---|---|---|
| | | *Days.* | | | *Days.* |
| No. 5 | F | 25–27 | No. 5 | F | 27–29 |

Mosquito No. 6 (Set 3, Group I) was made to sting at age periods of 2, 4, 6, 9, and 12 days longer than in the successful case reported by Marchoux and Simond. In all, five subjects were used.

TABLE 5.—*Inoculation with mosquito No. 6.*

[Nonimmunes are designated by capital letters.]

| Mosquito. | Stung nonimmune— | Age of mosquito at time of stinging. | Mosquito. | Stung nonimmune— | Age of mosquito at time of stinging. |
|---|---|---|---|---|---|
| | | *Days.* | | | *Days.* |
| No. 6 | F | 24 | No. 6 | J | 31 |
| No. 6 | G | 26 | No. 6 | M | 34 |
| No. 6 | H | 28 | | | |

Mosquitoes Nos. 7, 8, and 9 (Set 3, Group II) were made to sting subjects B and F. Subject B was stung by all three insects 3 (to 5) days after completing their metamorphoses. Subject F was stung by insect No. 9 on the twenty-fifth (to twenty-seventh) and again on the twenty-seventh (to twenty-ninth) day after its metamorphosis.

TABLE 6.—*Inoculation with mosquitoes Nos. 7, 8, and 9.*

[Nonimmunes are designated by capital letters.]

| Mosquito. | Stung nonimmune— | Age of mosquito at time of stinging. | Mosquito. | Stung nonimmune— | Age of mosquito at time of stinging. |
|---|---|---|---|---|---|
| | | *Days.* | | | *Days.* |
| Nos. 7, 8, and 9 | B | 3-5 | No. 9 | F | 27-29 |
| No. 9 | F | 25-27 | | | |

Mosquito No. 10 (Set 3, Group III) stung subjects B and G, 2 and 26 days respectively after emergence from the pupal shell, the latter inoculation being 4 days after the 22-day period.

TABLE 7.—*Inoculation with mosquito No. 10.*

[Nonimmunes are designated by capital letters.]

| Mosquito. | Stung nonimmune— | Age of mosquito at time of stinging. | Mosquito. | Stung nonimmune— | Age of mosquito at time of stinging. |
|---|---|---|---|---|---|
| | | *Days.* | | | *Days.* |
| No. 10 | B | 2 | No. 10 | G | 26 |

With mosquitoes Nos. 11, 12, 13, and 14 we made one inoculation at least 22 days after completing their metamorphoses, and nine inoculations of seven subjects (B, F, G, J, L, M, and N) at varying intervals after this period up to and including the fortieth day after attaining the adult stage, as shown in Table 8.

TABLE 8.—*Inoculation with mosquitoes Nos. 11, 12, 13, and 14.*

[Nonimmunes are designated by capital letters.]

| Mosquito. | Stung nonimmune— | Age of mosquito at time of stinging. | Mosquito. | Stung nonimmune— | Age of mosquito at time of stinging. |
|---|---|---|---|---|---|
| | | *Days.* | | | *Days.* |
| Nos. 11 and 12 | H | 22-27 | No. 11 | F | 32-37 |
| Nos. 13 and 14 | J | 23-28 | No. 12 | B | 32-37 |
| Nos. 11 and 12 | J | 25-30 | Nos. 12, 13, and 14 | G | 34-39 |
| No. 13 | G | 25-30 | Nos. 12 and 13 | J | 36-41 |
| No. 14 | [a] L | 26-31 | Nos. 13 and 14 | N | 39-44 |
| Nos. 12, 13, and 14 | [b] M | 30-35 | No. 12 | N | 40-45 |

[a] Subject passed from observation after 6 days.
[b] Subject passed from observation after 5 days.

We have recorded above our work with particular reference to the mosquitoes. Below (Table 9) we tabulate it with reference to the nonimmunes who were the subjects of the inoculations.

TABLE 9.—*Nonimmunes and inoculations to which they were subjected.*

| Subject, age, and nationality. | Stung by mosquito— | Age of mosquito at time of inoculation.[a] |
|---|---|---|
| | | *Days.* |
| A, nonimmune, 23 years, Canadian | No. 2 | 6 |
| | No. 2 | 12 |
| B, nonimmune, 44 years, Dalmatian | Nos. 6 and 10 | 2 |
| | Nos. 7, 8, and 9 | 3 |
| | No. 1 | 9 |
| | Nos. 3 and 4 | 13 |
| | Nos. 1 and 2 | 20 |
| | No. 12 | 32 |
| C, nonimmune, 16 years, Austrian | No. 2 | 10 |
| | Nos. 1 and 2 | 13 |
| D, nonimmune, 26 years, Norwegian | Nos. 1 and 2 | 24 |
| E, nonimmune, 21 years, American | No. 3 | 21 |
| F, nonimmune, 23 years, American | No. 6 | 24 |
| | Nos. 5 and 9 | 25 |
| | No. 3 | 26 |
| | Nos. 7 and 9 | 27 |
| | No. 11 | 32 |
| G, nonimmune, 23 years, American | No. 13 | 23 |
| | Nos. 6 and 10 | 26 |
| | Nos. 3 and 4 | 29 |
| | No. 2 | 32 |
| | Nos. 12, 13, and 14 | 34 |
| H, nonimmune, 34 years, American | Nos. 11 and 12 | 22 |
| | No. 6 | 28 |
| | No. 2 | 35 |
| J, nonimmune, 45 years, Irish | Nos. 13 and 14 | 23 |
| | Nos. 11 and 12 | 25 |
| | No. 6 | 31 |
| | No. 3 | 35 |
| | Nos. 12 and 13 | 36 |
| K, nonimmune, 27 years, Norwegian | No. 2 | 39 |
| L, nonimmune, 38 years, American | No. 14 | 26 |
| | No. 3 | 37 |
| M, nonimmune, 20 years, American | Nos. 12, 13, and 14 | 30 |
| | No. 6 | 34 |
| N, nonimmune, 31 years, Irish | Nos. 13 and 14 | 39 |
| | No. 12 | 40 |
| | No. 3 | 49 |

[a] Ages given in this table are minimal. For further details consult previous tables.

In all, thirteen subjects were used. After each inoculation they were kept under observation for at least 7 days, except in three instances, in two of which the observation period was 5 and in the third 6 days.

Our results were uniformly negative, no reaction of any kind being observed in any of the subjects of our inoculations.

## CONCLUSION.

In view of the negative results recorded by us in our efforts to confirm the positive work of Marchoux and Simond, we feel that additional work will be necessary to settle the question of the hereditary transmission of the parasite of yellow fever in the *Stegomyia fasciata*. Nevertheless, the sanitarian will do well to continue his measures of mosquito destruction after the suppression of an epidemic.

# APPENDIX.[a]

# THE HEREDITARY TRANSMISSION OF THE VIRUS OF YELLOW FEVER IN THE STEGOMYIA FASCIATA.

By E. Marchoux and P. L. Simond.

Among the new facts concerning yellow fever which we gathered in Brazil there is one the importance of which obliges us to publish at once. It concerns the question of the possibility of the hereditary transmission of the yellow fever virus from mosquito to mosquito.

Since 1903 our attention has been turned to the fact that in certain foci of an epidemic zone it is at times difficult to find a case of yellow fever of recent date as the origin of the new cases which spring up at a given time. There being no doubt that the lighting up of these foci was due to the presence of infected *Stegomyia fasciata*, we were forced to conclude that one or several of these mosquitoes had in some way been imported from a distant point where the disease existed from which they had drawn the virus. This undoubtedly occurs frequently. We, nevertheless, were led to ask ourselves if, under certain circumstances, eggs derived from a Stegomyia infected in the course of an epidemic some months prior to the one observed could not have given birth to Stegomyias hereditarily infected.

Several experiments were made in 1903 to confirm this hypothesis. We had some Stegomyias lay eggs that had stung some cases early in the disease. The eggs were hatched into larvæ, and the adult insect raised from these was made to sting a human subject.

These experiments did not give us positive results at that time, although the subjects that had been bitten by these mosquitoes were not immune to the disease, for subsequently it was possible to give it to them by injections of fresh virulent serum.

We resumed these experiments in February, 1905. We collected a number of eggs of one laying from a Stegomyia 20 days old which had stung several of our cases in order to determine a heavy infection, and the larvæ which were hatched the 4th of February were placed in a jar to be reared. The adult insects began to emerge February 16. These, isolated in tubes from the time of emergence, were fed with sirup until March 2. At this date, 14 days after the metamorphosis, two of these Stegomyia stung subject A, a Portuguese, who had arrived in Brazil a few days before, and had up to that time never had yellow fever. The subject showed no reaction following this inoculation.

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[a] Translated from Comptes Rendus, Société Biologie, Paris, Vol. LIX, No. 27. August 4, 1905, p. 259.

He was stung again by one of these two mosquitoes (the second having died in the meantime) on the 10th of March, 8 days after the first inoculation. Four days later, March 14, he developed a typical though mild attack of yellow fever. The character of the period of invasion, the vomiting, the pains, the course of the temperature, the icterus, and the progress of convalescence permitted no doubt as to the nature of the disease.

We deemed it a duty, nevertheless, to confirm our diagnosis experimentally. After recovering, this subject was twice submitted to the stings of several Stegomyia infected by a case of yellow fever. He showed himself absolutely refractory to these inoculations, as do all individuals recently immunized by a first attack.

Let us add that the conditions under which he was observed by us from the time of his arrival in Brazil do not permit that any source of contamination, other than the hereditarily infected mosquito by which he had been stung, could have brought on the attack of yellow fever which he had presented.

It may be concluded from this experiment that under conditions which can not as yet be precisely defined the *Stegomyia fasciata*, the progeny of a mother directly infected by a case of yellow fever, are themselves infected hereditarily. It follows from the various experiments done on this subject that the time needed by the mosquito hereditarily infected to become capable of discharging the virus with its salivary secretion is longer than in the case of the mosquito which has drawn the virus directly from the blood of a patient. This period was 22 days in the positive case.

It follows likewise, both from experiments and from epidemiologic facts, that this hereditary transmission can not be considered as the general rule but rather as an exceptional occurrence.

The mildness of the attack suffered by A warrants the belief that the passage of the virus from one generation of Stegomyia to another is accompanied by a certain amount of attenuation. This may be a new field open to research with reference to vaccination against yellow fever.

The knowledge of this mode of propagation explains one of the most obscure points in the history of yellow fever, that of the recurrence of certain epidemics under conditions where a primary case can not be found that is sufficiently recent to explain the infection of the Stegomyia.

Finally, its importance can not be disregarded from the point of view of prophylaxis.

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